Supplemental Materials

Supplemental Materials and Methods

Plasmid construction

pZK194 (pREP41-GFP-ypt5) was constructed as follows. The *ypt5*$^{+}$ ORF and terminator region (1 kb downstream from the ORF) were amplified by PCR using 5'-GTCAGGTCGAC(*Sal*I)AATGGCATCAAATACAGCT-3' and 5'-CATCGGAGCTC(*Sac*I)ATTGCGGAGAAGCCTCCTC-3' as forward and reverse primers, respectively. The underlined sequences indicate restriction enzyme sites. The PCR product was digested with *Sal*I and *Sac*I, and then inserted into *Sal*I- and *Sac*I-digested pTN54 (pREP41-GFP), yielding pZK194 (pREP41-GFP-ypt5).

Immunofluorescence microscopy

For cell fixation, we followed the method of Hagan and Hyams (1988) and used glutaraldehyde and paraformaldehyde. Gma12-HA was visualized by indirect immunofluorescence microscopy using rat anti-HA antibody 3F10 (Roche) and Alexa 594-conjugated goat anti-rat IgG (Molecular Probes, Eugene, OR). To visualize the nuclear chromatin region, cells were stained with DAPI at 1 μg/ml.

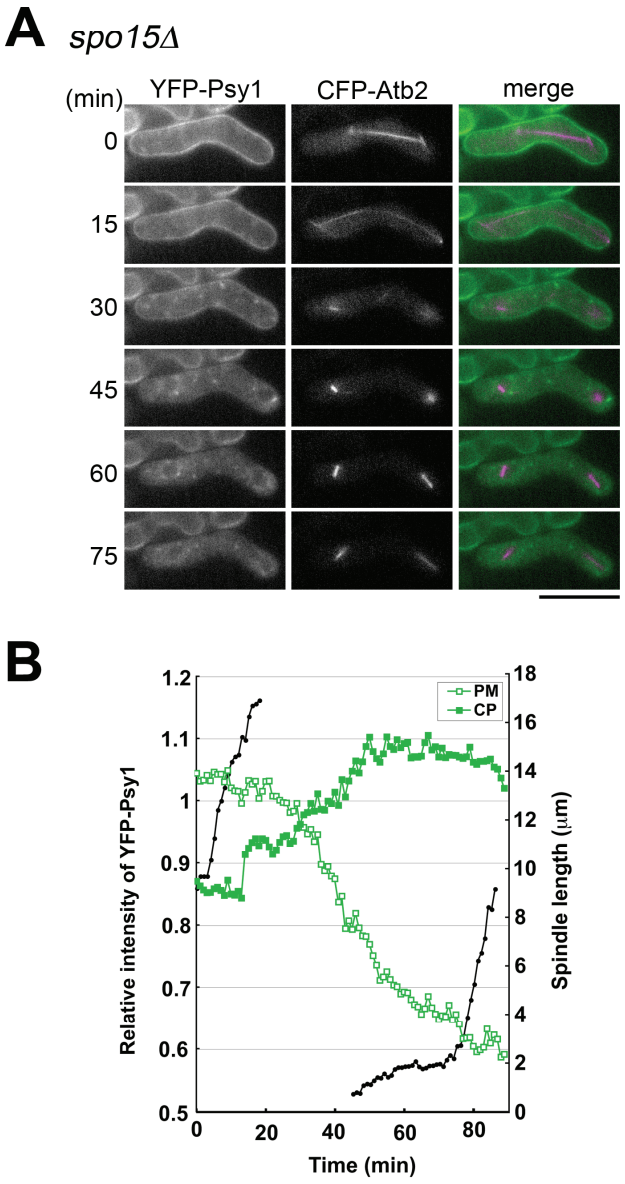
DRM isolation and immunoblotting

The DRM was isolated as described (Bagnat *et al.*, 2000). Cells were collected and washed with distilled water. The cell pellet was then suspended in 0.5 ml of TNE buffer [50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA]. After the addition of PMSF, the cells were ruptured with glass beads. The lysate was cleared by centrifugation at 500 x *g* for 5 min. A 0.3-ml aliquot of TNE buffer was added to 0.25 ml of the lysate, which was then incubated with Triton X-100 (1 % final) for 30 min on ice. After extraction of Triton X-100, the lysate (0.4 ml) was adjusted to 40% Optiprep by the addition of 0.8 ml of Optiprep solution (Axis-Shield, Oslo, Norway), and overlaid with 1.92 ml of 30% Optiprep in TXNE (TNE with 0.1% Triton X-100) and 0.32 ml of TXNE. The samples were centrifuged at 55,000 rpm for 2 h at 4˚C using an ultracentrifuge (CP70G, Hitachi, Tokyo, Japan), with a swing rotor (RPS65T, Hitachi) and 13 x 51 mm polycarbonate tubes (Hitachi), and six fractions of equal volume were collected from the top. The top fraction was subjected to a second incubation with Triton X-100, loaded onto a second Optiprep gradient, and centrifuged again. Fractions from the gradients were precipitated with 10% (w/v) TCA, dissolved in appropriate volumes of cracking buffer [8 M urea,

5% (w/v) SDS, 1 mM EDTA, 50 mM Tris/HCl (pH 6.8), 5% (v/v) 2-mercaptoethanol], and incubated at 65˚C for 20 min. Samples were resolved by SDS-PAGE on 10% gels and then transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were probed with a mouse anti-GFP antibody (Roche) at a 1:1000 dilution, and a rabbit polyclonal anti-Psy1 antibody (Maeda *et al.*, 2009) or a rabbit polyclonal anti-Spo14 antibody (Nakamura-Kubo *et al.*, 2003) at a 1:2000 or 1:500 dilution, respectively. Immunoreactive bands of GFP fusion proteins were visualized by staining with an ECL horseradish peroxidase-conjugated sheep anti-mouse IgG (GE Healthcare) at a 1:5000 dilution and ECL-plus detection reagent (GE Healthcare). Immunoreactive bands of Psy1 or Spo14 were visualized by staining with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) at a 1:5000 dilution and ECL detection reagent (GE Healthcare).

Supplementary Figure Legends

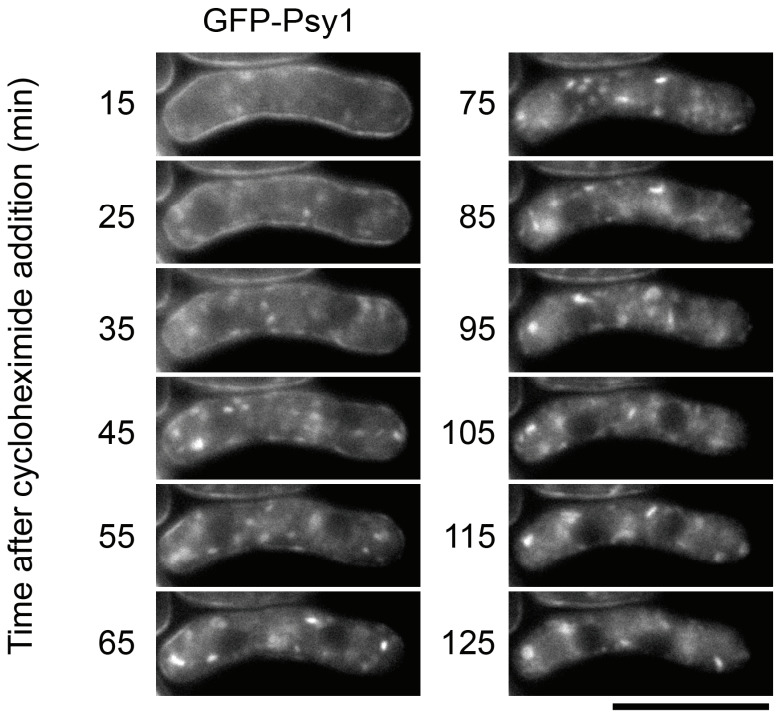
Supplemental Figure 1 Kashiwazaki et al. (2011)



Supplemental Figure S1. Internalization of Psy1 is not affected by a defect in FSM formation. (A) A *spo15Δ* strain (TN353) expressing YFP-Psy1 and CFP-Atb2 was cultured on SSA containing 2 μM thiamine at 28˚C for 16 hr. Images were obtained every 1 minute, and those taken at 15-minute intervals are shown (See also

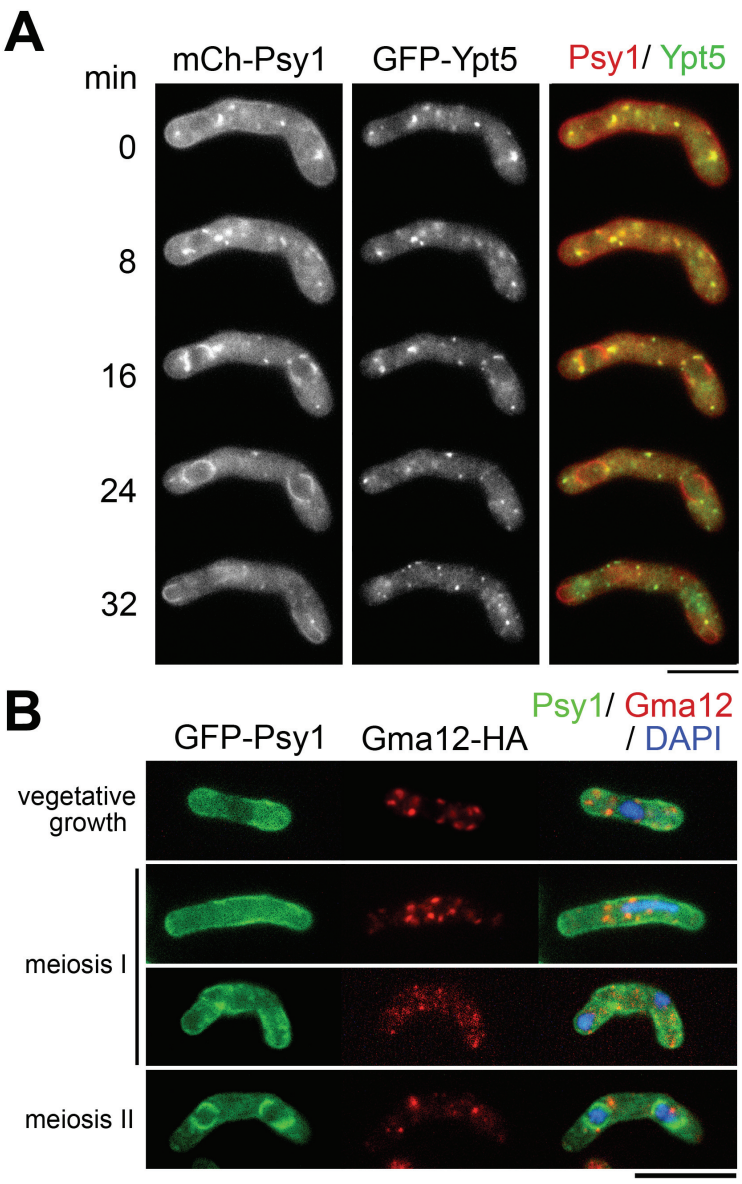
Supplemental Movie 7). Two images, YFP-Psy1 and CFP-Atb2, have been merged using two pseudocolors, green and magenta, respectively. Bar, 10 μm. (B) Kinetics of Psy1 internalization in *spo15Δ*. Measurements of the fluorescence intensity of YFP-Psy1, in addition to the length of spindle microtubules, were performed as described in Figure 1.

Supplemental Figure 2 Kashiwazaki et al (2011)



Supplemental Figure S2. Effect of cycloheximide on Psy1 internalization. A wild-type strain (YN68) expressing GFP-Psy1 was cultured on SSA at 28˚C for 16 hr. Cells at meiosis I were suspended in SSL-N liquid medium containing 200 μg/ml of cycloheximide for 15 min, after which images were obtained every 2 min. Images taken at 10-minute intervals are shown (See also Supplemental Movie 5). Bar, 10 μm.

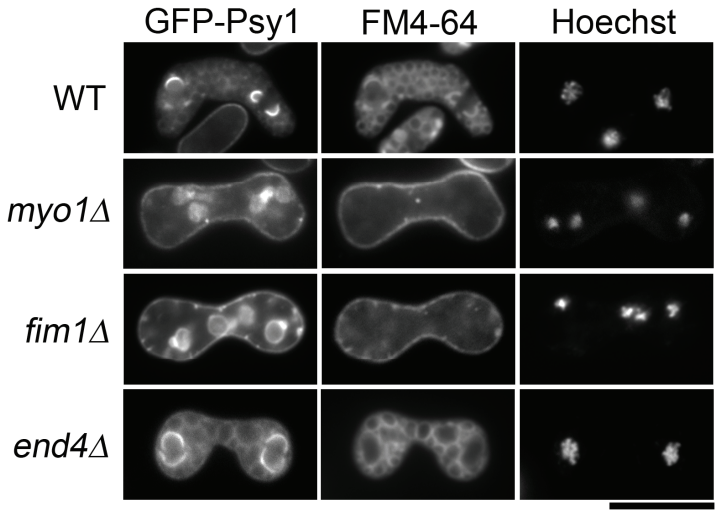
Supplemental Figure S3 Kashiwazaki et al (2011)



Supplemental Figure S3. After internalization, Psy1 is transported to the FSM via the endosome. (A) A wild-type strain (ZK151[pREP41-GFP-ypt5]) expressing mCherry-Psy1 and GFP-Ypt5 was cultured on SSA at 28˚C for 16 hr. Images of a cell just after meiosis I were obtained every minute and those taken at 8-minute intervals are shown (See also Supplemental Movie 6). Two images, mCherry-Psy1 and GFP-Ypt5,

have been merged using two pseudocolors, red and green, respectively. (B) A wild-type strain (ZK225[pREP1-gma12-HA]) expressing GFP-Psy1 and Gma12-HA was incubated on MM-N containing 0.05 μM thiamine at 28˚C for 16 hr. Gma12-HA and the chromatin region were visualized as described in Supplementary Materials and Methods. Bars, 10 μm.

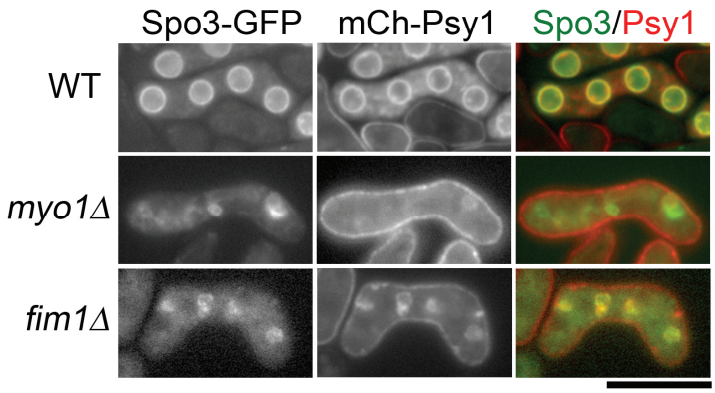
Supplemental Figure S4 Kashiwazaki et al (2011)



Supplemental Figure S4. Simultaneous observation of the behavior of GFP-Psy1 and the internalization of FM4-64.

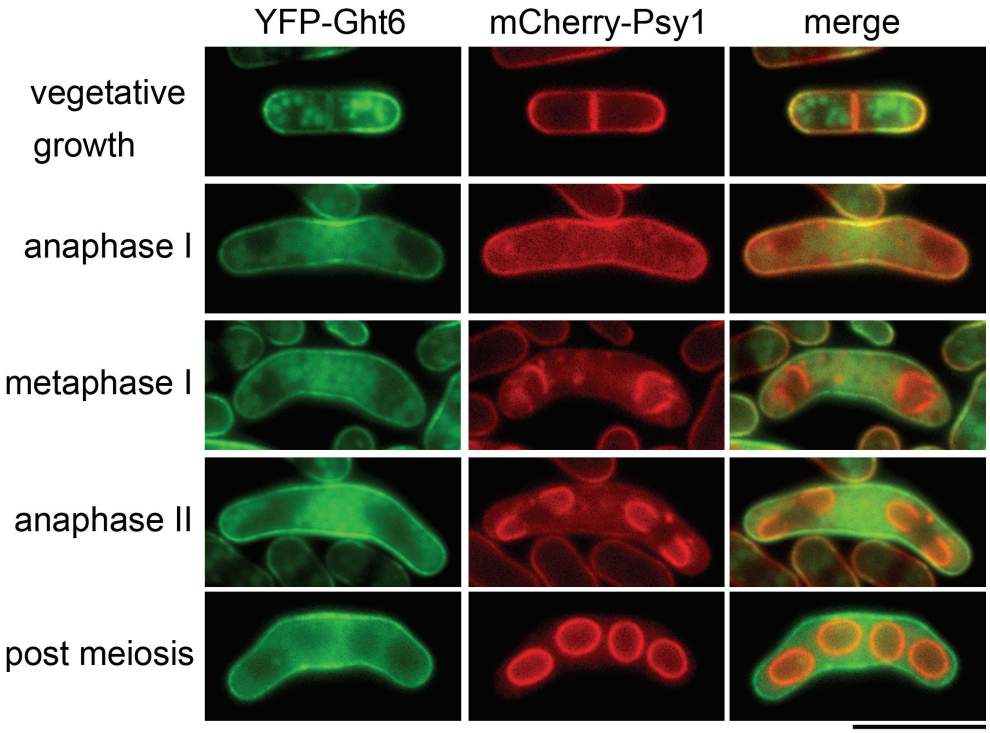
Wild-type (YN68), *myo1*Δ (ZK194), *fim1*Δ (ZK186), and *end4*Δ (ZK69) strains expressing GFP-Psy1 strains expressing GFP-Psy1 was cultured on SSA at 28˚C for 16 h. The chromatin region was stained with Hoechst 33342. Cells were then labeled with 8 μM FM4-64 for 5 min and observed immediately. Bar, 10 μm.

Supplemental Figure S5 Kashiwazaki et al (2011)



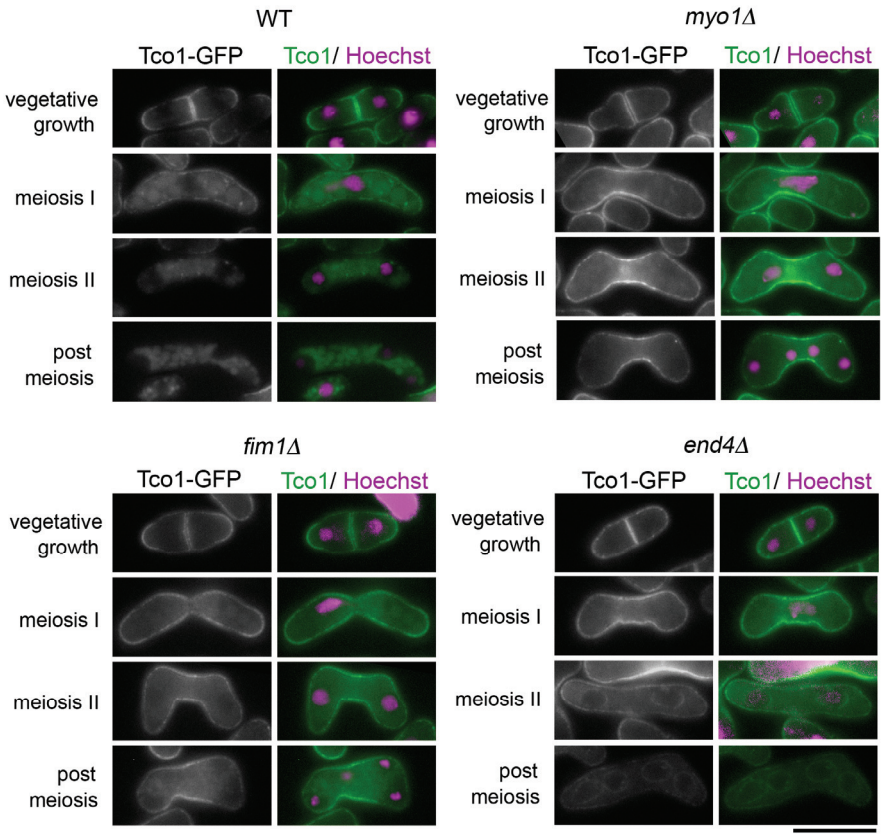
Supplemental Figure S5. Simultaneous observation of Spo3-GFP and mCherry-Psy1. Wild-type (TN454), *myo1*Δ (TN458), and *fim1*Δ (TN455) strains expressing Spo3-GFP and mCherry-Psy1 were cultured on SSA at 25˚C for 16 hr. Two images, Spo3-GFP and mCherry-Psy1, have been merged using two pseudocolors, green and red, respectively. Bar, 10 µm.

Supplemental Figure S6 Kashiwazaki et al (2011)



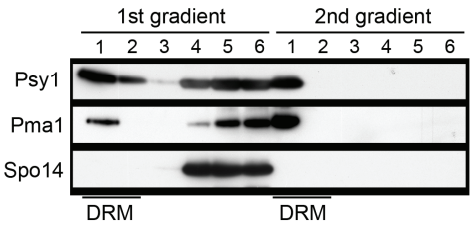
Supplemental Figure S6. Localization of Ght6 during meiosis. Wild-type strain (ZK230) expressing Ght6-YFP and mCherry-Psy1 was cultured on SSA at 25˚C for 16 hr. The chromatin region was stained with Hoechst 33342. Bar, 10 μm.

Supplemental Figure S7 Kashiwazaki et al (2011)



Supplemental Figure S7. Internalization of Tco1 during meiosis. Wild-type (ZK353), *myo1Δ* (ZK347), *fim1Δ* (ZK342), and *end4Δ* (ZK338) strains expressing Tco1-GFP were cultured on SSA at 25˚C for 16 hr. The chromatin region was stained with Hoechst 33342. Bar, 10 μm.

Supplemental Figure S8 Kashiwazaki et al (2011)



Supplemental Figure S8. Detergent solubility of GFP-Psy1 and Pma1-GFP in Optiprep gradients. Cells expressing Pma1-GFP (ZK216) were cultured in MM medium at 28˚C. Solubilization of Pma1-GFP was tested in Optiprep gradients. Fractions 1–6 (top to bottom of the gradient) were analyzed by Western blotting using anti-Psy1, anti-GFP, or anti-Spo14 antibodies to detect Psy1, Pma1, or Spo14, respectively. DRM, detergent-resistant membrane.

Supplementary Movie Legends

Supplemental Movie 1. Behavior of GFP-tagged Psy1 during sporulation in a wild-type cell. Wild-type strain YN68 was cultured on sporulation medium (SSA) at 28˚C for 16 h, stained with Hoechst 33342, and then grown in SSL-N medium. Time-lapse images of living zygote cells were acquired. The FSM and microtubule are shown in green and magenta, respectively.

Supplemental Movie 2. Psy1 internalizes at the interphase after meiosis I. The movie corresponds to the frames shown in Figure 1A. In the merged movie, the FSM and nucleus are shown in green and magenta, respectively.

Supplemental Movie 3. Psy1 is internalized normally in *spo15Δ*. The movie corresponds to the frames shown in Supplemental Figure 1A.

Supplemental Movie 4. Behavior of Dendra2-Psy1. The movie corresponds to the frames shown in Figure 1C.

Supplemental Movie 5. Cycloheximide treatment does not affect the internalization of Psy1. The movie corresponds to the frames shown in Supplemental Figure 2.

Supplemental Movie 6. Psy1 is internalized by endocytosis. The movie corresponds to the frames shown in Figure 2B.

Supplemental Movie 7. Psy1 is transported to the FSM via the endosome. The movie corresponds to the frames shown in Supplemental Figure 3A.

Supplemental Table 1. Strains used in this study.

Strain	Genotype	Source/Reference
15/A08-YFH	*h^{90} leu1<<P$_{nmt1}$-mug33-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
20/B06-YFH	*h^{90} leu1<<P$_{nmt1}$-sec9-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
25/G09-YFH	*h^{90} leu1<<P$_{nmt1}$-ght6-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
45/C01-YFH	*h^{90} leu1<<P$_{nmt1}$-bsu1-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
47/H02-YFH	*h^{90} leu1<<P$_{nmt1}$-cta3-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
50/G04-YFH	*h^{90} leu1<<P$_{nmt1}$-sod2-YFP-FLAG-His$_6$*	Matsuyama *et al.*, 2006
AI227 (FY21404)*	*h^{90} myo1::kan^r leu1-32*	This study
CP18-8 (FY13842)*	*h$^+$ cps8-188*	Ishiguro and Kobayashi, 1996
end4D (FY12693)*	*h^{90} end4::ura4$^+$ ura4-D18 leu1-32*	Iwaki *et al.*, 2004
erg2D	*h^{90} erg2::ura4$^+$ ura4-D18 leu1-32*	Iwaki *et al.*, 2008
ET21 (FY21405)*	*h^{90} mei4::ura4$^+$ pmd1::GFP kanr ura4-D18*	This study
ET22 (FY21406)*	*h^{90} mei4::ura4$^+$ tco1::GFP kanr ura4-D18*	This study
H05/C05 (FY15503)*	*h^{90} ght6::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H06/C07 (FY15465)*	*h^{90} phz1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H09/H11 (FY14815)*	*h^{90} SPBC1652.02::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H10/A12 (FY14959)*	*h^{90} ctr5::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H10/C09 (FY15020)*	*h^{90} cki3::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H10/H11 (FY14958)*	*h^{90} tco1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H12/H07 (FY14997)*	*h^{90} cki1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H16/D05 (FY15336)*	*h^{90} SPAC32A11.02c::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H17/C01 (FY15399)*	*h^{90} SPBC36.03c::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H17/C06 (FY15413)*	*h^{90} syp1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H17/F05 (FY15410)*	*h^{90} css1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
H17/G11 (FY15429)*	*h^{90} pmd1::GFP kanr ade6-M216 ura4-D18 leu1-32 lys1*	Hayashi *et al.*, 2009
JW393 (FY12684)*	*h^{90} myo1::kan^r ade6-M216 leu1-32*	Toya *et al.*, 2001
JW635 (FY12686)*	*h^{90} myo1-GFP::kan^r leu1-32*	M Yamamoto
KFM101 (FY17228)*	*h$^+$ fim1::ura4$^+$ ade6-M216 ura4-D18 leu1-32*	Nakano and Mabuchi, 2006
TN8 (FY7132)*	*h^{90} leu1-32*	Nakamura *et al.*, 2001
TN29 (FY7085)*	*h^{90} ura4-D18 leu1-32*	Nakase *et al.*, 2001
TN344 (FY13227)*	*h^{90} ade6<<YFP-psy1 leu1-32* [pREP1-CFP-atb2]	Nakamura *et al.*, 2008
TN353 (FY13234)*	*h^{90} spo15::ura4$^+$ ura4-D18 ade6<<YFP-psy1 leu1-32* [pREP1-CFP-atb2]	Nakamura et al, 2008
TN454 (FY21358)*	*h^{90} ade6<<mCherry-psy1 spo3-GFP<<LEU2*	This study
TN455 (FY21359)*	*h^{90} fim1Δ:: ura4$^+$ ade6<<mCherry-psy1 spo3-GFP<<LEU2*	This study
TN458 (FY21360)*	*h^{90} myo1Δ::kan^r ade6<<mCherry-psy1 leu1<<spo3-GFP*	This study
YN68 (FY12296)*	*h^{90} leu1<<GFP-psy1*	Nakase *et al.*, 2004
YW917 (FY7513)*	*h^{90} mei4::ura4$^+$ ade6-M216 ura4-D18 leu1-32*	Horie *et al.*, 1998
ZK69 (FY21361)*	*h^{90} end4::ura4$^+$ ura4-D18 leu1<<GFP-psy1*	This study
ZK118 (FY21362)*	*h^{90} cps8-188 leu1<<GFP-psy1*	This study
ZK121 (FY21363)*	*h^{90} ura4-D18 leu1<<GFP-psy1*	This study
ZK146 (FY21364)*	*h^{90} myo1-GFP::kan^r leu1<<mCherry-psy1*	This study
ZK151 (FY21365)*	*h^{90} ade6<<mCherry-psy1 leu1-32*	This study
ZK184 (FY21366)*	*h^{90} fim1::ura4$^+$ ade6-M216 ura4-D18 leu1<<GFP-psy1*	This study
ZK185 (FY21367)*	*h^{90} fim1::ura4$^+$ ura4-D18 leu1-32*	This study
ZK186 (FY21368)*	*h^{90} fim1::ura4$^+$ ura4-D18 leu1<<GFP-psy1*	This study
ZK194 (FY21369)*	*h^{90} myo1::kan^r leu1<<GFP-psy1*	This study
ZK196 (FY21370)*	*h^{90} myo1::kan^r ade6-M216 leu1<<GFP-psy1*	This study
ZK207 (FY21371)*	*h^{90} end4::ura4$^+$ ade6-M216 ura4-D18 leu1<<GFP-psy1*	This study
ZK216 (FY21372)*	*h^{90} leu1<<pma1-GFP*	This study
ZK217 (FY21373)*	*h^{90} ade6<<mCherry-psy1 leu1<<pma1-GFP*	This study
ZK225 (FY21374)*	*h^{90} ade6<<GFP-psy1 leu1-32*	This study
ZK228 (FY21375)*	*h^{90} ade6<<mCherry-psy1 leu1<<P$_{nmt1}$-mug33-YFP-FLAG-His$_6$*	This study

ZK230 (FY21376)*	h^{90} *ade6<<mCherry-psy1 leu1<<*P_{nmt1}*-ght6-YFP-FLAG-*His_6	This study
ZK232 (FY21377)*	h^{90} *ade6<<mCherry-psy1 leu1<<*P_{nmt1}*-bsu1-YFP-FLAG-*His_6	This study
ZK233 (FY21378)*	h^{90} *ade6<<mCherry-psy1 leu1<<*P_{nmt1}*-cta3-YFP-FLAG-*His_6	This study
ZK234 (FY21379)*	h^{90} *ade6<<mCherry-psy1 leu1<<*P_{nmt1}*-sod2-YFP-FLAG-*His_6	This study
ZK237 (FY21380)*	h^{90} *erg2::ura4*$^+$ *ura4-D18 leu1<<GFP-psy1*	This study
ZK247 (FY21381)*	h^{90} *leu1<<Dendra2-psy1*	This study
ZK256 (FY21382)*	h^{90} *mei4::ura4*$^+$ *ade6<<GFP-psy1 ura4-D18 leu1-32*	This study
ZK264 (FY21383)*	h^{90} *myo1::kanr ade6<<GFP-psy1 leu1-32*	This study
ZK270 (FY21384)*	h^{90} *fim1::ura4*$^+$ *ade6<<GFP-psy1 ura4-D18 leu1-32*	This study
ZK276 (FY21385)*	h^{90} *end4::ura4*$^+$ *ade6<<GFP-psy1 ura4-D18 leu1-32*	This study
ZK338 (FY21386)*	h^{90} *end4::ura4*$^+$ *tco1::GFP kanr ura4-D18*	This study
ZK339 (FY21387)*	h^{90} *end4::ura4*$^+$ *pmd1::GFP kanr ura4-D18*	This study
ZK342 (FY21388)*	h^{90} *fim1::ura4*$^+$ *tco1::GFP kanr ura4-D18*	This study
ZK343 (FY21389)*	h^{90} *fim1::ura4*$^+$ *pmd1::GFP kanr ura4-D18*	This study
ZK347 (FY21390)*	h^{90} *myo1::kanr tco1::GFP kanr*	This study
ZK348 (FY21391)*	h^{90} *myo1::kanr pmd1::GFP kanr*	This study
ZK352 (FY21392)*	h^{90} *SPBC1652.02::GFP kanr*	This study
ZK353 (FY21393)*	h^{90} *tco1::GFP kanr*	This study
ZK354 (FY21394)*	h^{90} *pmd1::GFP kanr*	This study
ZK371 (FY21395)*	h^{90} *ade6<<mCherry-psy1 leu1-32* [pAI1]	This study
ZK372 (FY21396)*	h^{90} *ade6<<mCherry-psy1 leu1-32* [S644]	This study
ZK373 (FY21401)*	h^{90} *ade6<<mCherry-psy1 leu1-32* [TN46]	This study
ZK374 (FY21402)*	h^{90} *ade6<<mCherry-psy1 leu1-32* [TC45]	This study
ZK375 (FY21403)*	h^{90} *ade6<<mCherry-psy1 leu1-32* [pZK183]	This study

*YGRC, Yeast Genetic Resource Center Japan (http://yeast.lab.nig.ac.jp/nig/). The strains constructed in this study will be deposited in the YGRC.

Supplemental Table 2. Plasmids used in this study

Name	Description	Selection Markers	Reference
pAI1	pAL-8C9.04c-GFP	*amp; LEU2*	A Itadani
pAL-KS (FYP788)*	*ars1*	*amp; LEU2*	Tanaka *et al.*, 2000
pREP1-gma12-HA	pREP1-gma12-HA	*amp; LEU2*	T Yoko-o
pREP41	*ars1, nmt41* promoter	*amp; LEU2*	Maundrell, 1993
S644 (FYP1836)*	pEG3-*pck1*(1- aa)-GFP	*amp; LEU2*	Ding *et al.*, 2000
TN46 (FYP1891)*	pEG3-*wsc1*(1-280 aa)-GFP	*amp; LEU2*	Ding *et al.*, 2000
TC45 (FYP1991)*	pEG3-*23B6*(1- aa)-GFP	*amp; LEU2*	Ding *et al.*, 2000
pTN54	pREP41-GFP	*amp; LEU2*	Nakamura *et al*, 2001
pTN143 (FYP410)*	pAL-KS-GFP-*nmt1* terminator	*amp; LEU2*	Ikemoto *et al.*, 2000
pTN363 (FYP872)*	pBR322-*leu1*-P_{psy1}-GFP-*psy1*	*amp; leu1*$^+$	Nakamura-Kubo et al., 2003
pTN381 (FYP890)*	pBR322-*leu1*	*amp; leu1*$^+$	Kashiwazaki *et al.*, 2005
pTN459 (FYP969)*	pREP1-CFP-*atb2*	*amp; LEU2*	Nakamura *et al*, 2008
pTN505 (FYP1014)*	pIA-YFP-*psy1*	*amp; ade6*$^+$	Nakamura *et al*, 2008
pYN113 (FYP135)*	pREP41(*ade6*)-*mei4*	*amp; ade6*$^+$	Y Nakese
pZK182	pIA-mCherry-*psy1*	*amp; ade6*$^+$	This study
pZK183	pREP41-GFP-*sec1*	*amp; LEU2*	This study
pZK188	pAL-*pma1*-GFP	*amp; LEU2*	This study
pZK194	pREP41-GFP-*ypt5*	*amp; LEU2*	This study
pZK200	pTN381-*pma1*-GFP	*amp; leu1*$^+$	This study
pZK205	pREP2-GFP-*ypt5*	*amp; ura4*$^+$	This study
pZK206	pBS-Dendra2	*amp*	This study
pZK207	pBS-Dendra2-*psy1*	*amp*	This study
pZK208	pBS-P_{psy1}-Dendra2-*psy1*	*amp*	This study
pZK209	pTN381-P_{psy1}-Dendra2-*psy1*	*amp; leu1*$^+$	This study

*YGRC, Yeast Genetic Resource Center Japan (http://yeast.lab.nig.ac.jp/nig/). The Plasmids constructed in this study will be deposited in the YGRC.

Supplementary References

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